

### **REMARKS**

Claims 28-41 are now pending, with Claim 28 being the sole independent claim. Claims 42-48 are nonelected and therefore have been cancelled without prejudice or disclaimer of the subject matter recited therein. Applicants retain the right to pursue the nonelected subject matter in a divisional application or applications pursuant to 35 USC §121.

The priority statement has been amended to include the PCT application information.

Hyperlinks have been deleted from the specification and amino acid sequences on pages 35-36 have been identified by SEQ ID NO. identifier. The nonelected inventions have been removed from claims 32 and 33 and thus should be allowable.

Regarding claim rejections under 35 USC 112, first paragraph (enablement). Applicants respectfully traverse. Applicants would like to bring examiner's attention to Example 8 of the above identified application. Example 8 discloses two Regions (1 and 2) in the monofunctional corn and E.coli aspartate kinases "where particular amino acid substitution were known to yield lysine-insensitive monofunctional E.coli aspartate kinase (see U.S. Patent 5,773,691)". Attached hereto as Appendix A1, is a comparison of the E.coli (gi:7428061) and the corn monofunctional aspartate kinase (SEQ ID NO:6). The two Regions, mentioned in Example 8, are shown underlined on Appendix A1 and are substantially conserved among the corn and the E.coli aspartate kinases. Amino acids identical among the two sequences are indicated with an asterisk (\*) on the top row; the program to maximize alignment of the sequences uses dashes. Residues that upon mutation rendered the aspartokinases from corn and E.coli insensitive to feedback regulation are indicated by a dot below the residue. In Region 1 the E.coli lysine-insensitive aspartate kinase has the T (threonine) residue changed to I (isoleucine) and in Region 2 the M (methionine) residue changed to I (isoleucine). Furthermore this invention discloses a site-specific mutation in the corn monofunctional aspartate kinase (S (serine) to L (leucine)) at the position in Region 1 where a T (threonine) residue was changed to I (isoleucine). This change rendered the corn monofunctional aspartate kinase insensitive to feedback regulation by lysine (Example 8, page 37, line 4-12). In view of the foregoing Applicants believe that the specification sufficiently enables any person skilled in the art to make and use the invention commensurate in scope with the claims and provides sufficient enablement for sequences having less than 100% sequence identity compared to the claimed aspartate kinase sequence (SEQ ID NO:6). Withdrawal of the rejection under 35 USC, first paragraph is therefore respectfully requested.


Regarding rejection of claims under 35 USC 112, first paragraph (written description), Applicants respectfully traverse. First, Applicants submit that the specification discloses to one of ordinary skill in the art a representative number of aspartate kinases with at least 80% sequence identity to SEQ ID NO:6, and not just a single polynucleotide encoding SEQ ID NO:6.

The specification at page 9, line 31 through page 10, line 6 discloses alterations in nucleotide sequence that are not expected to alter functionality, such as alterations that produce a chemically equivalent amino acid at a given site or alterations in the N- or C-terminal portions. Thus, from the foregoing, the skilled artisan would immediately understand the specification to disclose a representative number of polynucleotide sequences, having different nucleotide substitutions, that encode aspartate kinase but that vary (within 80% sequence identity) of SEQ ID NO:6. Since SEQ ID NO:6 and the E.coli sequence share only 27% identity, one of skill in the art would have appreciated that many variants sharing at least 80% sequence identity to the SEQ ID NO:6 would have been expected to retain aspartate kinase activity. Indeed, introduction of the E.coli monofunctional feedback insensitive aspartate kinase into canola led to increased lysine production, confirming the expectation that aspartate kinases from divergent species are functionally similar (Falco et al. Biotechnology (1995) 13:577-582, copy enclosed together with a supplemental IDS).

Applicants believe that the foregoing is responsive to each of the points recited in the Office Action, and submit that the present application is in allowable form. Favorable consideration and passage to issue are solicited.

Please charge any requisite fee to Deposit Account No. 04-1928 (E. I. du Pont de Nemours and Company).

Respectfully submitted,

  
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APPENDIX A1

SEQ ID NO:6 MAIPVRSAAAPRRLVPSIPPASSGHVRGLACFGTRTGPRGARGLSMVVADSTSRRAKQAD  
GI:7428061 MSE-----  
\* \* \* \* \*

SEQ ID NO:6 GGDGVLGAPVLGGLGMEGLGDQLSVVMKFGGSSVSSAARMAEVAGLILTFPEERPVVVLS  
GI:7428061 -----IVVSKFGGTSVADFDAMNRSADIVLSDANVR-LVVLS  
\* \* \* \* \*

SEQ ID NO:6 AMGKTTNNLLLLAGEKAVGCGVIHVSEIEEWNMVKSLHIKTVDLGLPXICNTSLYELEQL  
GI:7428061 ASAGITNLLVALAE-----GLEPGERFEKLDAIRNIQFALERLRYPNVIRE--EIERL  
\* \* \* \* \*

SEQ ID NO:6 LKGIAMMKELT-----PRTSDYLVSFGECEMSTRIFSAYLNKIRVKARQYDAFDIGFITTD  
GI:7428061 LENITVLAEAAAALATSPALTDDELVSHGELMSTLLFVEILRERDVQAQWFDVRKV-MRTND  
\* \* \* \* \*

SEQ ID NO:6 EFGNADILEATYPAVAKRLHGDWIDPAIPVVTGFLGKGWKSAGVTTLGRGGSDDLATTI  
GI:7428061 RFGRAEPDIAALAEALQLLPRLNE-GLVITQGFIGSE-NKGRTTTLGRGGSDDYTAALL  
\* \* \* \* \*

SEQ ID NO:6 GKALGLREIQVWKDVGVLTCDPNIYPHAKTVPYLTFFEEATELAYFGAQLHPQSMRPAR  
GI:7428061 AEALHASRVDIWTDPGIYTTDPRVVSAAKRIDEIAFAEAAEMATFGAKVLHPATLLPAV  
\* \* \* \* \*

SEQ ID NO:6 EGDIPVRVKNSYNPKAPGTLITRQDMDXGLVVLTSIVLKSNTMLDIVSTRMLGQYGF  
GI:7428061 RSDIPVFGSSKDPAGGTLVCNKTENPP---LFRALALRRNQTLTLHSLNMLHSRGFL  
\* \* \* \* \*

Region 2

SEQ ID NO:6 ARVSGICYIEDLCISVDCVATSEVSVSVSLDPSKIWSRELIQQASELDHVVEELEKIAIV  
GI:7428061 AEVFGI--LARHNISVDLITTTSEVSVALTLDTT---GSTSTGDTLLTQSLLMELSALCRV  
\* \* \* \* \*

Region 1

SEQ ID NO:6 RLLQQRAIISLIGN-VEQSSLILEKTGRVLRKSGVNVQMISQGASKVNMSLIVHDSAKA  
GI:7428061 EVEEGLALVALIGNDLKACGVGKEVFGVL-EPFNIRMICYGASSHNLCLVPGEDAEQ  
\* \* \* \* \*

SEQ ID NO:6 LVEALHQAFFEDDVLSQVEAENLLVG  
GI:7428061 VVQKLHNSLFE-----  
\* \* \* \* \*